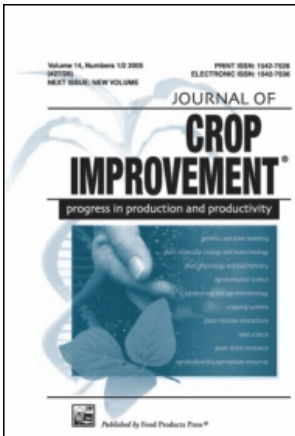

Journal of Crop Improvement
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t792303981

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Online publication date: 06 November 2010

To cite this Article Williams, W. Paul , Windham, Gary L. , Krakowsky, Matthew D. , Scully, Brian T. and Ni, Xinzhi(2010) 'Aflatoxin Accumulation in BT and Non-BT Maize Testcrosses', Journal of Crop Improvement, 24: 4, 392 — 399
To link to this Article: DOI: 10.1080/15427528.2010.505111
URL: http://dx.doi.org/10.1080/15427528.2010.505111

Journal of Crop Improvement, 24:392–399, 2010
ISSN: 1542-7528 print/1542-7535 online
DOI: 10.1080/15427528.2010.505111

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Aflatoxin Accumulation in BT and Non-BT Maize Testcrosses

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The accumulation of aflatoxin, produced by the fungus Aspergillus flavus *in maize, is a chronic problem in the southeastern United States. Its presence in grain greatly reduces its value and marketability. Aflatoxin accumulation is frequently associated with high temperatures, drought, and insect damage. Ten maize germplasm lines, some selected for resistance to aflatoxin accumulation, were crossed to transgenic (transformed with genes from* Bacillus thuringiensis *Berliner (BT) and expressing the Cry1Ab protein) and non-transgenic versions of LH287. Testcrosses were evaluated for ear damage from insect feeding and aflatoxin accumulation. Ear damage caused by insect feeding and aflatoxin accumulation was significantly less in BT than non-BT testcrosses. The germplasm line × BT/non-BT interaction was not significant. Three lines selected for resistance to aflatoxin accumulation (Mp313E, Mp717, Mp04:97) exhibited the lowest levels of aflatoxin*

This manuscript is a contribution of USDA-ARS in cooperation with the Mississippi Agricultural and Forestry Experiment Station. It is published as Journal No. J-11836 of the Mississippi Agricultural and Forestry Experiment Station.

Appreciation is extended to Monsanto Company, St. Louis, MO, for providing seed for making testcrosses.

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whether crossed to the BT or non-BT versions of LH287. Neither the levels of aflatoxin accumulation nor ear damage differed significantly between the BT and non-BT versions of these hybrids. This indicates that adding BT to hybrids produced from Mp313E, Mp717, or Mp04:97 would not be expected to increase resistance to aflatoxin accumulation.

KEYWORDS Aflatoxin, Aspergillus flavus, Bacillus thuringiensis, maize

INTRODUCTION

Mycotoxins such as aflatoxins and fumonisins occur naturally in maize, *Zea mays* L., and cause major food and feed safety concerns. The accumulation of aflatoxin, produced by the fungus *Aspergillus flavus* in maize, is a chronic problem in the southeastern United States. Aflatoxin is a potent carcinogen (Castegnaro & McGregor 1998; Pittet 1998). It is toxic to livestock, pets, and wildlife (Gourama & Bullerman 1995; Leung, Diaz-Liano, & Smith 2006). The presence of aflatoxin in grain substantially reduces its value and marketability.

Accumulation of high levels of aflatoxin in maize is frequently associated with high temperatures and drought; the problem is further exacerbated by insects feeding on the developing ears. Although growing hybrids with resistance to *A. flavus* infection and aflatoxin accumulation is generally considered an important strategy in reducing aflatoxin contamination of maize, commercial hybrids with adequate levels of resistance for high-stress environments are not available. Researchers at several public institutions have, however, identified and developed germplasm lines as sources of resistance to aflatoxin contamination (Betràn et al. 2005; Clements & White 2005; Williams 2006; Williams, Windham, & Buckley 2008).

Aflatoxin accumulation is highly sensitive to environmental effects. The variability associated with environments and significant genotype × environment interactions are major impediments to identifying and developing new sources of resistance to *A. flavus* infection and aflatoxin accumulation. Even in areas where aflatoxin contamination is a chronic problem, relying on natural infection is not adequate for effective germplasm screening. Inoculating developing ears with *A. flavus* has been used to facilitate germplasm screening and reduce variation within experiments across locations and years (Zummo & Scott 1989; Windham et al. 2005).

Damage from ear-feeding insects such as southwestern corn borer, *Diatraea grandiosella* Dyar; fall armyworm, *Spodoptera frugiperda* (J.E. Smith); and corn earworm, *Helicoverpa zea* Boddie, is frequently associated with increased levels of aflatoxin accumulation (Dowd, Johnson, & Williams

2005). Differences in levels of insect damage among locations and years and even among plots within an experiment can contribute to significant genotype × environment interactions and further complicate the evaluation of maize germplasm for resistance to aflatoxin accumulation. Growing hybrids transformed with genes from *Bacillus thuringiensis* Berliner (BT) has proved to be effective in reducing levels of aflatoxin accumulation in environments favorable to fungal infection and aflatoxin accumulation and where populations of ear-feeding insects are high (Williams et al. 2002, 2005).

This investigation was undertaken to compare ear damage and aflatoxin accumulation among single crosses produced by crossing 10 germplasm lines with varying degrees of resistance or susceptibility to aflatoxin accumulation to BT (expressing the Cry1Ab insecticidal protein) and non-BT versions of LH287. A second objective was to determine whether using a BT line, rather than a non-BT line, when producing seed of testcrosses would be more effective in screening germplasm for resistance to aflatoxin accumulation.

MATERIALS AND METHODS

Ten maize germplasm lines were crossed with LH287 and LHBT1-1 to produce 10 pairs of BT and non-BT testcrosses. LH287 and LH 287BT1-1 were provided by Monsanto Company, St. Louis, MO. LH287BT1-1 had been transformed with the *Cry1Ab* gene. The 10 lines used in making the testcrosses represented varying levels of resistance and susceptibility to aflatoxin accumulation: Mp313E, Mp420, and Mp717 were developed and released as sources of resistance to aflatoxin accumulation (Scott & Zummo 1990, 1992; Williams & Windham 2006); Mp04:97, Mp04:107, and Mp04:110 are breeding lines selected for resistance to aflatoxin accumulation; and B73, GA209, SC212m, and Va35 are susceptible to aflatoxin accumulation (Williams 2006; Williams, Windham, & Buckley 2008).

The 20 testcrosses, which comprised a 2 × 10 factorial set of treatments, were planted in single-row plots in a randomized complete-block design with four replications at three locations in 2009: Raleigh, North Carolina; Tifton, Georgia; and Starkville, Mississippi. Standard production practices were followed at each location.

Seven days after silks had emerged from 50% of the plants in a plot, the top ear of each plant was inoculated with *A. flavus* isolate NRRL3357, which is known to produce aflatoxin, using the side-needle technique (Zummo & Scott 1989). With this technique, a tree-marking gun fitted with a 14-gauge hypodermic needle was used to inject a 3.4-ml suspension containing 3×10^8 *A. flavus* conidia in sterile distilled water underneath the husks

into the side of the ear. Inoculum was prepared as described by Windham & Williams (2002).

At maturity, 10 ears per plot were hand harvested. Insect damage was determined by the procedure described by Widstrom (1967) for quantifying corn earworm damage. The depth of penetration into the tip of the ear was measured in centimeters. Afterwards, the ears were bulked and shelled. The grain was thoroughly mixed and ground using a Romer mill (Union, MO). The concentration of aflatoxin in a 50-g sample was determined using the Vicam Afla test (Watertown, Massachusetts), a procedure that detects aflatoxin at levels as low as 1 ng/g.

The data on ear damage and aflatoxin concentration were analyzed using the GLM procedure of SAS (SAS Institute 2003). Aflatoxin values were transformed as ln(y+1), where y is the concentration of aflatoxin in a sample before statistical analysis. The transformation was performed to provide a more nearly normal distribution. Data for both traits were analyzed both within and across locations. Means were compared using Fisher's protected least significant difference (LSD) at $P = 0.05$ (Steel & Torrie 1980).

RESULTS AND DISCUSSION

The analysis of variance of ear damage rating indicated that locations, germplasm lines, and BT/non-BT were highly significant sources of variation (Table 1). The location × germplasm line and location × BT/non-BT interactions were also significant, but the germplasm line × BT/non-BT interaction was not significant.

TABLE 1 Analysis of Variance for Ear Damage and Aflatoxin Concentration in Maize Hybrids Produced by Crossing Germplasm Lines to LH287 and LH287BT and Evaluated at Raleigh, NC; Tifton, GA; and Starkville, MS, in 2009

		Mean squares	
Source	df	Ear damage	Aflatoxin ln(y+1)
Locations	2	45.33**	0.41
Reps (locations)	9	0.21	0.97
Germplasm line	9	1.30**	37.83**
BT/non-BT	1	6.06**	5.89**
Germplasm line × BT/non-BT	9	0.49	1.38
Location × germplasm line	18	1.24**	2.61**
Location × BT/non-BT	2	2.18**	0.05
Locations × germplasm line × BT/non-BT	18	0.61	0.77
Error	170	0.48	0.72

**Significant at $P < 0.01$.

TABLE 2 Ear Damage and Aflatoxin Accumulation in Corn Hybrids Produced by Crossing 10 Germplasm Lines to Non-BT and BT Versions of LH287 and Evaluated in Raleigh, NC; Tifton, GA; and Starkville, MS, in 2009

			Aflatoxin(ng g^{-1})[b]			
	Ear damage[a]		LH287		LH287BT	
Germplasm Line	LH287[c]	LH287BT[c]	Ln(y+1)[c]	Geometric mean	Ln(y+1)[c]	Geometric mean
SC212m	1.64 ab	1.05 bc*	7.20 ab	1343	7.67 a	2134
GA209	1.20 bc	1.00 bc	7.59 a	1981	6.74 bc*	843
B73	1.77 a	1.78 a	7.08 ab	1184	7.02 ab	1123
Va35	1.57 ab	0.88 bc*	6.66 bc	779	6.31 c	551
Mp04:107	1.47 abc	1.18 bc	6.10 cd	443	6.22 c	501
Mp04:110	0.91 c	0.87 bc	5.51 de	247	5.11 d	165
Mp420	1.53 ab	0.74 c*	5.52 de	248	4.84 de*	88
Mp04:97	1.36 abc	1.31 ab	4.99 ef	147	4.28 e	71
Mp313E	1.18 bc	1.04 bc	4.08 g	58	4.25 e	69
Mp717	1.38 abc	0.98 bc	4.35 fg	77	3.83 e	45
Mean	1.40	1.09*	5.91		5.58*	
LSD(0.05)	0.56	0.56	0.68		0.68	

[a]Ear damage as indicated by depth (cm) of feeding penetration into the tip of the ear at harvest.
[b]Values for aflatoxin accumulation were transformed [ln(y+1)] before statistical analysis. Geometric means were obtained by reverse transformation to the original units of measurement.
[c]Values in a column followed by the same letter do not differ at $P = 0.05$ (Fisher's Protected LSD).
*Indicates significant differences between non-BT and BT versions of hybrids.

Mean ear damage for the three locations was 1.40 for non-BT hybrids and 1.09 for BT hybrids, respectively (Table 2). Within locations, the means for non-BT and BT hybrids were 0.57 and 0.16 for North Carolina, 1.76 and 1.72 for Georgia, and 1.92 and 1.32 for Mississippi. The difference between BT and non-BT hybrids was significant for North Carolina and Mississippi, but not for Georgia. Within both the LH287 and LH287BT hybrids, B73 sustained the highest level of ear damage. Three germplasm lines, SC212m, Va35, and Mp420, sustained significantly heavier damage when crossed with LH287 than LH287BT (Table 2). Differences among the locations may have been due, at least in part, to the species of insects present. European corn borer (*Ostrinia nubilalis* Hubner) is a frequent pest of maize in North Carolina, whereas southwestern corn borer is found in Mississippi, but not in North Carolina or Georgia. Corn earworm occurs in all three locations, but it is generally more prevalent in Georgia and Mississippi than North Carolina.

The analysis of variance of aflatoxin accumulation indicated that germplasm lines, BT/non-BT, and the location × germplasm line interaction were highly significant sources of variation (Table 1). None of the other main effects or interactions was a significant source of variation.

Mean aflatoxin accumulation levels for the three locations were 369 ng g^{-1} for non-BT hybrids and 266 ng g^{-1} for BT hybrids. Within

locations, mean aflatoxin accumulation for non-BT and BT hybrids were 382 ng g^{-1} and 249 ng g^{-1} for North Carolina, 398 ng g^{-1} and 287 ng g^{-1} for Georgia, and 332 ng g^{-1} and 259 ng g^{-1} for Mississippi. The correlation between the 10 germplasm lines crossed to LH287 and LH287BT was positive and highly significant ($r^2 = 0.90$, $P < 0.0001$). Although overall aflatoxin accumulation was significantly lower when germplasm lines were crossed to LH287BT than LH287, the difference for individual germplasm lines crossed to LH287BT and LH287 was significant for only GA209 and Mp420 (Table 2). For the other germplasm lines, BT had no effect on aflatoxin accumulation. Aflatoxin accumulation was lowest for Mp04:97, Mp313E, and Mp717 whether crossed to LH287 or LH287BT. Mp313E and Mp717 were developed and released as sources of resistance to aflatoxin accumulation (Scott & Zummo 1990; Williams & Windham 2006). The other line released as a source of resistance to aflatoxin accumulation, Mp420 (Scott & Zummo 1992), was one of two lines that exhibited significantly lower levels of aflatoxin accumulation when crossed to LH287BT than when crossed to LH287. Mp420 also sustained less ear damage when crossed to LH287BT. Apparently, the reduced ear damage allowed the resistance to aflatoxin accumulation inherent in Mp420 to be expressed in Mp420 × LH287BT.

Although germplasm lines and BT/non-BT effects were highly significant sources of variation for both ear damage and aflatoxin accumulation, their interaction was not significant (Table 1). This would indicate that, at least among this group of lines, either LH287 or LH287BT could be effectively used in making testcrosses to evaluate for resistance to aflatoxin accumulation. The level of ear damage was low for BT and non-BT hybrids. In years or locations where ear damage is heavy, resistance to insect damage could be more important in reducing aflatoxin accumulation than in the current investigation. Under such conditions, evaluating germplasm for resistance to *A. flavus* infection and aflatoxin in testcrosses with BT lines could enhance the likelihood of identifying useful sources of resistance.

CONCLUSIONS

Although testcrosses with LH287BT sustained less ear damage and accumulated lower levels of aflatoxin than testcrosses with LH287, both lines were effective in identification of germplasm with resistance to aflatoxin contamination. SC212m, GA209, B73, and Va35 exhibited the highest levels of aflatoxin contamination whether crossed to LH287 or LH287BT. Mp313E, Mp717, and Mp04:97 exhibited the highest levels of resistance to *A. flavus* infection and aflatoxin accumulation when crossed to either LH287 or LH287BT. These germplasm lines should be useful in maize breeding programs with the objective of developing maize hybrids with resistance to

A. flavus infection and aflatoxin accumulation. The superiority of the BT crosses over non-BT crosses was not apparent in this investigation. This could be the result of relatively low insect pressure at the three locations. In situations where insect pressure is high, differences between BT and non-BT crosses would likely be greater. In such situations, the resistance from these lines used in combination with BT should maximize protection against aflatoxin contamination.

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